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Amycolatopsis methanolica sp. nov., a Facultatively Methylotrophic Actinomycete

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The generic position of a gram-positive, facultatively methylotrophic actinomycete known as *Nocardia* sp. strain 239 was determined by comparing reverse transcriptase sequences of 16S rRNA. The assignment of the organism to the genus *Amycolatopsis* was strongly supported by chemotaxonomic and morphological data. A comparison with the type strains of validly described *Amycolatopsis* species showed that the organism formed the nucleus of a new species. The name proposed for this new species is *Amycolatopsis methanolica*. The organism has been deposited in the National Collection of Industrial Bacteria as NCIB 11946.

Methanol-utilizing bacteria that assimilate formaldehyde via the ribulose monophosphate cycle (1, 42) are potential vehicles for fermentative overproduction of aromatic amino acids (7, 38, 40). The precursors of the shikimate pathway in these strains, namely, erythrose-4-phosphate and phosphoenolpyruvate, are intermediate and end products, respectively, of the ribulose monophosphate cycle. Gram-negative ribulose monophosphate cycle bacteria are not amenable to the extensive physiological and genetical manipulations needed for strain development in view of their obligate methylotrophic nature (26). There is, however, evidence that gram-positive, facultatively methylotrophic bacteria (6, 8) are suitable for strain improvement studies (4, 35).

The single gram-positive ribulose monophosphate cycle actinomycete already described was initially labeled *Streptomyces* sp. strain 239 (29–31) and then *Nocardia* sp. strain 239 (24). Stable mutants have been isolated from this metabolically versatile organism, which has been grown under diverse conditions in batch and continuous cultures (4, 24). Regulation of aromatic amino acid biosynthesis in the strain has been studied in detail (5), and examination of the systematic deregulation of these control systems and the development of a transformation system are under way. Preliminary chemosystematic studies included in this report showed that *Nocardia* sp. strain 239 has chemical properties consistent with its assignment of the family *Pseudonocardiaceae* (11), which encompasses the genera *Actinopolyspora*, *Amycolatopsis*, *Faenia*, *Pseudonocardia*, *Saccharomonospora*, *Saccharopolyspora*, and in all probability, *Amycolata* (T. Bowen, E. Stackebrandt, M. Dorsch, and M. Embley, J. Gen. Microbiol., in press). In the present investigation, *Nocardia* sp. strain 239 was further characterized and designated the type strain of *Amycolatopsis methanolica* sp. nov.

MATERIALS AND METHODS

Test strains and cultivation conditions. *Nocardia* sp. strain 239 (strain LMD 80.32 [Laboratory of Microbiology, Technical University of Delft, The Netherlands; NCIB 11946]) was the subject of chemotaxonomic, molecular systematic, and microbiological tests. The type strains of *Amycolata autotrophica* (K402; DSM 43210), *Amycolata hydrocarbonoxydans* (K428; DSM 43281), *Amycolata saturnea* (A195; DSM 43195), *Amycolatopsis azurea* (K114; J. Lacey, Rothamsted Experimental Station, Harpenden, United Kingdom), *Amycolatopsis fastidiosa* (K110; JCM 3275), *Amycolatopsis mediterranei* (K98, JCM 4789), *Amycolatopsis orientalis* (K99; JCM 4235), *Amycolatopsis rugosa* (K431; DSM 43194), *Amycolatopsis sulphurea* (K406; ATCC 27624), *Faenia rectivirgula* (F1; ATCC 33515), *Pseudonocardia thermophila* (G37; ATCC 19285), *Saccharomonospora viridis* (K73; ATCC 15386), and *Saccharopolyspora hirsuta* (K16; NCIB 11079) were included in all or some of the microbiological tests. Some of these organisms were also included in molecular systematic studies (10, 11; Bowen et al., in press), together with the type strains of *Actinopolyspora halophila* (K91; ATCC 27976), *Kibdelosporangium aridum* (K601; ATCC 39323), *Saccharopolyspora erythraea* (K600; Northern Research and Development Division, U.S. Department of Agriculture, Peoria, Ill.; NRRL 2338), and *Saccharothrix australiensis* (K409; NRRL 11239). The organisms were maintained on modified Bennett agar (28) and as suspensions of spores or mycelial fragments in glycerol (20%, vol/vol) stored at −20°C.

Biomass for the chemical analyses was prepared by growing *Nocardia* sp. strain 239 in shake flasks of modified Sauton broth (39) for 10 days at 30°C. Cultures were checked for purity, killed by shaking with Formalin (1%, vol/vol), harvested by centrifugation, washed with distilled water, and freeze-dried. Unless otherwise stated, the microbiological tests were incubated at 30°C (mesophilic strains) or 45°C (thermophilic strains; see Table 2) for 3 weeks with glucose-yeast extract agar (23) as the basal medium.

Wall sugar and amino acid analyses. Freeze-dried biomass (50 mg) defatted with tetrabutylammonium hydroxide at 100°C for 45 min was centrifuged, and the resultant pellet was washed in water, chloroform-methanol (2:1, vol/vol), and again in water. The isomeric form of diaminopimelic acid was determined by chromatography of defatted whole-organism hydrolysates on thin-layer sheets (Eastman Kodak 13255) as described by Staneck and Roberts (46). Defatted biomass was also hydrolyzed as described by Gilbart et al. (16) before analysis of whole-organism sugars as their alditol acetates by a modification of the method of Englyst and Cummings (13). The analysis was done with a Supelco SP-2330 (12 m by 0.25 mm [inside diameter]) capillary

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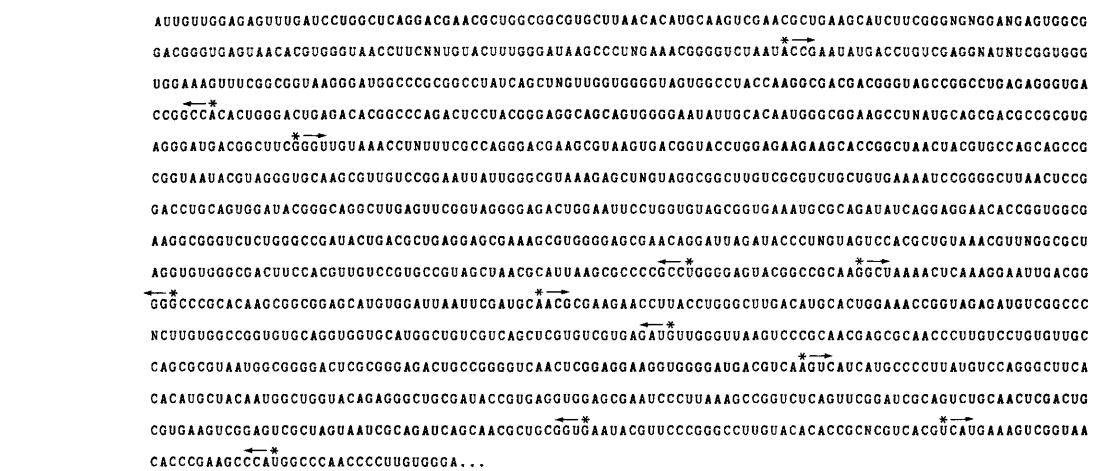

FIG. 1. Partial 16S rRNA sequence of *Nocardia* sp. strain 239. N, Unsequenced nucleotide. The regions enclosed by stars and arrows were used for calculation of homology values.

column fitted to a Packard model 427 chromatograph with nitrogen as the carrier gas, and the oven temperature was kept at 150°C for 8 min after injection and then raised 8°C/min to 240°C.

Extraction and analysis of fatty acid methylesters. Dried biomass (30 mg) was degraded by alkaline methanolysis (43), and analytical and preparative thin-layer chromatography of the methanolysates was performed as described previously (36, 41). Capillary gas chromatography of fatty acid methylesters was done as described by Saddler et al. (43). The retention times and relative proportions of the fatty acid methylesters were determined with a Shimadzu CR3A computing integrator. The identities of individual esters were established by comparison of their retention times with those of fatty acids extracted from *Saccharopolyspora hirsuta* K16 (12) and those of a standard fatty acid mixture (Supelco Inc.; catalog no. 4-7080).

Extraction and analysis of polar lipids and isoprenoid quinones. Dried biomass (50 mg) was examined by using the small-scale procedure of Minnikin et al. (37). Purified isoprenoid quinones were analyzed by electron impact mass spectrometry (2) on an AEI MS9 instrument with an ionizing voltage of 70 eV and a temperature range of 170 to 220°C. Polar lipid patterns were obtained by using previously published procedures (37, 49).

Extraction of cellular RNA, reverse transcriptase sequencing, and analysis of data. The procedure described by Embley et al. (10) was used for reverse transcriptase sequencing. K_{nuc} values (nucleotide substitution rate or evolutionary distance values of Hori [27]) were calculated, and an unrooted phylogenetic tree was generated by using the algorithm of Fitch and Margoliash (16) contained in the program written by Felsenstein (14) [PHYLIP version 2] for the IBM personal computer.

Biochemical tests. Esculin and arbutin hydrolyses were determined as described by Williams et al. (51), nitrate reduction was determined as described by Gordon and Mihm (22), and allantoinase and urease activities were determined as described by Gordon (20).

Degradation tests. Adenine (0.4%, wt/vol), casein (1.0%, wt/vol), elastin (0.3%, wt/vol), hypoxanthine (0.4%, wt/vol), testosterone (1.0%, wt/vol), tyrosine (0.4%, wt/vol), and xanthine (0.4% wt/vol) breakdown was detected in the basal medium; clearing of insoluble compounds from under and around areas of growth was scored as positive. Production of esterases able to breakdown glycerol tributyrate was detected in tributyrin agar (Oxoid Ltd.). DNA (0.2%, wt/vol) and starch (2%, wt/vol) degradation was recorded after 10 days in Bacto-DNase Test (Difco Laboratories) and glucose-yeast extract agars, respectively, by flooding plates with 1 M HCl and iodine solution (3) as appropriate and scoring zones of clearing as positive.

Enzyme tests. The test organisms were examined for the ability to cleavage 4-methylumbelliferone (4MU)- and 7-amino-4-methylcoumarin (7AMC)-conjugated fluorogenic substrates (see Table 2) by using the procedure of Slifkin and Gil (45) as modified by Goodfellow et al. (18, 19). Positive reactions were recorded when intense, light-blue fluorescence was observed under UV light at 366 nm.

Morphology and pigmentation. Diffusible and substrate mycelial pigments were recorded by eye on modified Bennett agar plates (28). The presence, amount, and color of aerial growth and spore chain morphology were observed on Czapeck Dox agar (Oxoid) with a binocular microscope (Nikon Kogaku K.K., Tokyo, Japan) at ×400 magnification. Spores of *Nocardia* sp. strain 239 were collected by pressing glass cover slips (12-mm diameter) gently onto a sporulating culture grown for 3 weeks on Czapeck Dox agar. The cover slips were attached to electron microscope stubs (14-mm diameter), the surface spore layers were coated with gold with a Nanotech splutter coater, and specimens were examined with a JEOL JSM-51 scanning electron microscope.

The micromorphology of *Nocardia* sp. strain 239 was determined with a culture grown at 30°C for 10 days (44). Preparations of whole cells were stained with aqueous malachite green (5%, wt/vol) and safranin (0.5%, vol/vol) and examined under a light microscope at ×1,000. Electron micrographs of ultrathin sections of spores (×190,000) were made with material fixed first in glutaraldehyde (6%, wt/vol) and 0.1 M cacodylate buffer at pH 7.2 for 30 min at 0°C and then in osmium tetraoxide (1%, wt/vol) and potassium chromate (2.5%, wt/vol) in the same buffer for 45 min at room temperature. The resultant preparations were dehydrated in a graded alcohol series, embedded in Epon 812, cut with a diamond knife, and examined in a Philips EM300 without further staining.

Nutritional tests. Strains were examined for the ability to grow on sole sugar and other carbon compounds as sources for energy and growth (see Table 2) by using the carbon-free medium of Stevenson (47). They were also inoculated onto

TABLE 1. Homology values for 490 selected nucleotides of the 16S rRNA catalogs of *Nocardia* sp. strain 239 and representatives of the genera *Actinopolyspora*, *Amycolata*, *Amycolatopsis*, *Faenia*, *Kibdelosporangium*, *Pseudonocardia*, *Saccharomonospora*, *Saccharopolyspora*, and *Saccharothrix*

No. and organism	% Homology with organism no.:										
	1	2	3	4	5	6	7	8	9	10	11
1. *Nocardia* sp. strain 239											
2. *Amycolatopsis fastidiosa* K110	93.6										
3. *Amycolatopsis azurea* K114	93.3	91.8									
4. *Saccharothrix australiensis* K409	91.0	90.0	90.4								
5. *Kibdelosporangium aridum* K601	92.0	91.8	92.4	91.1							
6. *Pseudonocardia thermophila* G37	90.3	89.9	89.8	90.5	91.6						
7. *Amycolata autotrophica* K402	90.9	89.2	89.6	89.3	91.7	91.8					
8. *Saccharopolyspora erythraea* K600	89.0	89.6	91.3	88.3	91.8	89.9	88.0				
9. *Saccharopolyspora hirsuta* K16	91.2	90.7	90.6	89.5	92.0	90.3	89.9	93.5			
10. *Faenia rectivirgula* F1	90.6	90.7	90.4	90.8	91.7	91.4	90.2	91.4	93.2		
11. *Saccharomonospora viridis* K73	89.4	89.5	89.4	87.6	90.5	89.3	87.9	88.4	88.6	91.1	
12. *Actinopolyspora halophila* K91	85.9	85.4	86.0	85.3	86.0	85.5	85.9	85.5	85.8	87.6	85.4

this medium alone (negative control) and onto this medium plus glucose (1%, wt/vol) (positive control). A positive result was recorded when growth was greater than that in the negative control.

Organisms were also examined for the capacity to grow in agarose mineral medium [K_2HPO_4, 5 g; $NaH_2PO_4 \cdot H_2O$, 1.5 g; $(NH_4)_2SO_4$, 1 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; carbon source, 10 mM; trace elements (48), 0.2 ml; agarose (Type 1, low EEO; Sigma Chemical Co., St. Louis, Mo.), 15 g/liter] containing a range of amino acids and C_1 compounds as sole sources of carbon (see Table 2).

Tolerance tests. Strains were examined for the ability to grow at pHs 5 and 6 at 20, 25, 30, 37, 45, 50, 55, and 60°C after 4 weeks and at 10°C after 6 weeks; visible growth was scored as a positive result. Growth in the presence of chemical inhibitors (see Table 2) was recorded as positive when it was greater than that in the negative control. Organisms were also screened for the ability to grow in glucose-yeast extract agar supplemented with antibiotics (see Table 2). Growth on test plates was compared with that on the basal medium alone, the control plate. Strains were scored as resistant when growth was equal or greater than that on the control plate.

Additional tests. Previously described methods (21) were used to determine the ability of *Nocardia* sp. strain 239 to produce acid from carbohydrates and to decarboxylate organic acids. The latter were added to agarose mineral medium to which 20 ml of a 0.04% solution of phenol red was added; utilization of the organic acids was established by the alkali-induced color of the phenol red after 28 days of incubation at 37°C. Similarly, carbohydrates (30 mM) were added to agarose mineral medium with phosphates at a third of the concentration given above; the pH of the medium was adjusted to 7.0 before addition of 15 ml of a 0.04% solution of bromocresol purple. Sloped cultures of the carbohydrate agars were observed for acid-induced color of the indicator after 28 days of incubation at 37°C. The test strain was also examined for its ability to grow in a rich medium (nutrient broth, 8 g; glycerol, 70 ml; agarose, 15 g; distilled water, 1 liter [pH 7.0]) supplemented with lysozyme (0.005%, wt/vol) as described by Gordon et al. (21) and for its capacity to grow on mineral medium broth supplemented with sole carbon sources (20 mM) within 28 days at 37°C.

RESULTS AND DISCUSSION

The partial reverse transcriptase sequence of *Nocardia* sp. strain 239 is shown in Fig. 1. Homology values based on the 490 nucleotides used to determine intragroup relationships (Table 1) were used to calculate K_{nuc} values. The phylogenetic tree generated from these values is depicted in Fig. 2. It is evident that *Nocardia* sp. strain 239 is closely related to *Amycolatopsis azurea* K114 and *Amycolatopsis fastidiosa* K110, especially to the latter. Classification should reflect genomic relationships, whereas taxonomies based on nucleic acid sequencing data should show phenotypic consistency (50). It is therefore significant that both the chemical and microbiological data support the assignment of the test strain to the genus *Amycolatopsis* (34).

Nocardia sp. strain 239 contains *meso*-diaminopimelic acid as a wall diamino acid; arabinose and galactose as wall sugars (i.e., it has wall chemotype IV and whole-organism sugar pattern type A sensu Lechevalier and Lechevalier [33]); and a phospholipid pattern consisting of diphosphatidylglycerol, phosphatidylethanolamine (taxonomically significant nitrogenous phospholipid), phosphatidylglycerol, phosphatidylinositol, phosphatidylmethylethanolamine, phosphatidylinositol mannosides, and two ninhydrin-positive glycophospholipids without phosphatidylcholine or phospholipids containing glucosamine (i.e., phospholipid pattern type 2

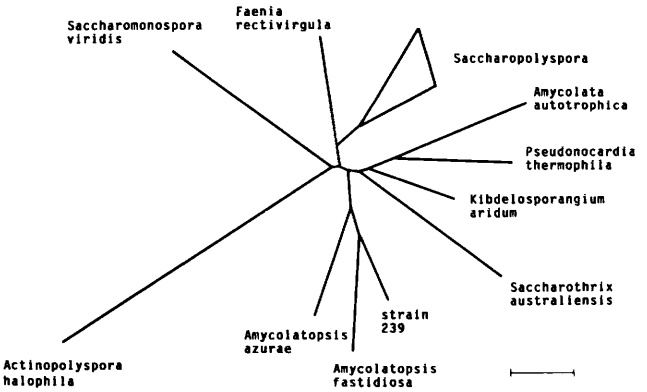

FIG. 2. Unrooted phylogenetic tree showing the relationships between *Nocardia* sp. strain 239 and the reference strains based on K_{nuc} values. Bar, 0.02 K_{nuc}.

TABLE 2. Properties of *Amycolatopsis methanolica* 239 compared with those of representative strains of the genera *Amycolata*, *Amycolatopsis*, *Faenia*, *Pseudonocardia*, *Saccharomonospora*, and *Saccharopolyspora*[a]

Test	*Amycolata autotrophica* K402	*Amycolata hydrocarbonoxydans* K428	*Amycolata saturnea* A195	*Amycolatopsis azurea* K114	*Amycolatopsis fastidiosa* K110[b]	*Amycolatopsis mediterranei* K98	*Amycolatopsis methanolica* 239	*Amycolatopsis orientalis* K99	*Amycolatopsis rugosa* K431	*Amycolatopsis sulphurea* K406	*Faenia rectivirgula* F1[b]	*Pseudonocardia thermophila* G37	*Saccharomonospora viridis* K73	*Saccharopolyspora hirsuta* K16
Biochemical tests														
Allantoin hydrolysis	+	−	+	+	−	−	−	+	+	−	+	−	+	+
Arbutin hydrolysis	+	−	−	+	+	+	−	+	+	−	+	−	−	+
Aesculin hydrolysis	+	+	+	+	−	+	+	+	+	−	+	+	−	+
Nitrate reduction	+	+	+	+	+	+	+	+	−	+	+	+	+	+
Urea hydrolysis	+	−	+	+	+	+	−	+	+	−	−	−	−	+
Degradation tests														
Adenine	+	−	−	ND[c]	ND	−	−	−	−	−	−	−	−	+
Casein	−	−	−	+	+	+	+	+	+	+	+	+	+	+
DNA	+	−	+	+	+	+	+	+	+	+	+	+	+	+
Elastin	−	−	−	−	+	+	+	−	−	−	−	−	+	+
Hypoxanthine	+	−	+	−	−	+	+	+	+	+	+	+	−	+
Starch	+	+	−	−	−	−	−	−	−	−	−	−	+	+
Testosterone	−	−	−	+	+	+	+	−	−	−	+	+	+	+
Tributyrin	+	−	+	+	−	+	−	+	+	−	+	−	+	+
Tyrosine	+	−	+	+	+	+	+	+	+	+	+	+	+	+
Xanthine	−	−	+	−	−	−	−	−	+	−	−	−	−	+
Cleavage of 4MU substrates														
Glycosides														
4MU-2-acetoamido-2-deoxy-β-D-galactopyranoside	−	+	−	+	−	−	+	−	−	−	−	−	−	−
4MU-2-acetamido-2-deoxy-β-D-glucopyranoside	−	+	−	+	+	−	+	+	+	+	+	−	+	+
4MU-*N*-acetyl-β-D-glucosaminide	−	+	−	+	+	−	+	+	+	+	+	−	+	+
4MU-α-L-arabinofuranoside	−	−	−	−	−	+	−	−	+	+	−	−	+	−
4MU-α-L-arabinopyranoside	−	−	−	−	−	−	−	−	−	−	−	−	+	−
4MU-α-D-cellobiopyranoside	−	−	−	−	−	+	−	−	−	−	−	−	−	−
4MU-β-D-fucopyranoside	+	+	+	+	+	−	−	−	+	+	−	+	−	−
4MU-α-D-galactopyranoside	−	−	−	−	−	+	−	+	−	−	−	−	−	−
4MU-β-D-galactopyranoside	−	+	−	+	+	+	−	+	+	−	−	+	−	−
4MU-α-D-glucopyranoside	+	−	+	−	+	+	−	+	−	+	−	−	+	−
4MU-β-D-glucopyranoside	+	+	+	+	−	+	+	+	+	−	−	−	−	+
4MU-β-D-glucuronide	−	−	−	−	−	−	−	−	−	−	−	−	−	−
4MU-β-D-xyloside	−	−	+	−	+	+	−	+	+	−	−	−	+	+
Inorganic esters														
4-MU-phosphate (acid)	−	+	+	+	+	+	+	+	+	+	+	+	+	+
4-MU-P_i	−	−	−	+	−	+	−	+	−	−	−	−	−	+
Organic esters														
4MU-heptanoate	+	+	+	+	+	+	+	+	+	−	+	+	+	+

Continued on following page

TABLE 2—*Continued*

Test	*Amycolata autotrophica* K402	*Amycolata hydrocarbonoxydans* K428	*Amycolata saturnea* A195	*Amycolatopsis azurea* K114	*Amycolatopsis fastidiosa* K110[b]	*Amycolatopsis mediterranei* K98	*Amycolatopsis methanolica* 239	*Amycolatopsis orientalis* K99	*Amycolatopsis rugosa* K431	*Amycolatopsis sulphurea* K406	*Faenia rectivirgula* F1[b]	*Pseudonocardia thermophila* G37	*Saccharomonospora viridis* K73	*Saccharopolyspora hirsuta* K16
4MU-laurate	+	+	+	+	+	+	+	+	−	+	−	−	+	+
4MU-nonanoate	+	+	+	+	+	+	+	+	−	+	−	+	+	+
4MU-palmitate	+	+	+	+	−	−	−	+	−	+	−	+	+	+
Cleavage of 7AMC substrates														
Endopeptidase substrates														
CBZ-arginine-7AMC	+	+	−	+	−	−	+	+	+	−	−	−	+	+
CBZ-arginine-arginine-7AMC	−	−	−	−	−	+	−	+	−	−	−	+	−	+
CBZ-glycine-glycine-leucine-7AMC	−	−	−	−	−	−	−	+	−	−	−	−	−	−
CBZ-phenylalanine-arginine-7AMC	−	−	−	−	−	+	+	+	−	−	−	+	−	+
CBZ-glutaryl-glycine-glycine-phenylalanine-7AMC	−	−	−	+	+	+	+	+	−	−	−	−	−	−
Succinate-leucine-tyrosine-7AMC	−	−	−	+	+	+	+	+	−	−	−	−	−	−
Exopeptidase substrates														
β-Alanine-7AMC	−	−	−	−	+	−	−	+	−	−	−	−	−	−
D-Alanine-7AMC	+	+	−	+	+	+	−	+	+	+	−	−	+	−
L-Alanine-7AMC	+	+	+	+	+	+	+	+	+	+	−	+	+	+
Arginine-7AMC	−	−	−	−	+	+	−	+	−	−	−	+	+	+
Z-arginine-7AMC	−	−	−	−	−	−	−	+	−	−	−	+	+	+
Aspartate-7AMC	−	−	−	−	−	+	−	−	−	−	−	−	−	−
S-benzylcysteine-7AMC	+	+	+	+	+	+	−	+	+	+	−	−	+	+
Glutamate-7AMC	−	−	−	−	+	+	−	+	−	−	−	−	+	−
Glutamate-7AMC-OH	−	−	−	−	+	−	−	−	−	−	−	−	−	+
Glycine-7AMC	−	−	−	−	+	−	−	+	−	−	−	+	−	−
Leucine-7AMC	+	+	+	−	+	+	−	+	−	−	−	+	−	−
Isoleucine-7AMC	−	−	−	+	+	+	+	+	+	+	+	+	−	−
Lysine-7AMC	−	−	−	−	−	+	−	+	−	−	−	+	+	+
Methionine-7AMC	−	−	−	−	+	+	−	+	−	−	−	+	+	+
Ornithine-7AMC	+	+	+	+	+	−	−	−	+	+	−	+	−	−
Pyroglutamate-7AMC	+	−	−	−	−	−	−	+	−	−	−	−	−	−
Serine-7AMC	−	+	+	+	+	+	−	+	−	−	−	+	+	+
Threonine-7AMC	+	−	−	−	−	+	−	+	−	−	−	−	−	−
Tyrosine-7AMC	+	+	+	+	+	+	+	+	+	+	−	+	+	+
Valine-7AMC	+	−	+	−	+	+	−	+	−	−	−	+	−	−
Morphology and pigmentation														
Color of substrate mycelium														
Brown	+	−	−	−	−	−	−	+	−	−	+	+	−	−
Cream	−	−	−	+	+	−	−	−	−	−	−	−	−	−
Fawn-buff	−	−	−	−	−	−	−	−	−	+	−	−	−	+

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TABLE 2—*Continued*

Test	*Amycolata autotrophica* K402	*Amycolata hydrocarbonoxydans* K428	*Amycolata saturnea* A195	*Amycolatopsis azurea* K114	*Amycolatopsis fastidiosa* K110[b]	*Amycolatopsis mediterranei* K98	*Amycolatopsis methanolica* 239	*Amycolatopsis orientalis* K99	*Amycolatopsis rugosa* K431	*Amycolatopsis sulphurea* K406	*Faenia rectivirgula* F1[b]	*Pseudonocardia thermophila* G37	*Saccharomonospora viridis* K73	*Saccharopolyspora hirsuta* K16
Green	−	−	−	−	−	−	−	−	−	−	−	−	+	−
Yellow	−	+	+	−	−	+	+	−	+	−	−	−	−	−
Diffusible pigment	−	−	−	+	−	−	−	−	−	+	−	−	+	−
Color of diffusible pigment														
Green	−	−	−	−	−	−	−	−	−	−	−	−	+	−
Pink	−	−	−	+	−	−	−	−	−	−	−	−	−	−
Tan	−	−	−	−	−	−	−	−	−	+	−	−	−	−
Aerial mycelium														
Present	+	+	+	+	−	−	+	+	−	+	+	+	+	−
Sparse	−	+	−	+	−	−	−	−	−	+	+	+	+	−
Abundant	+	−	+	−	−	−	+	+	−	−	−	−	−	−
Presence of spores on:														
Aerial mycelium	+	+	+	+	+	+	+	+	−	+	+	+	+	+
Substrate mycelium	−	−	−	−	−	−	−	−	−	−	+	−	−	−
Spore chain morphology														
Monosporate	−	−	−	−	−	−	−	−	−	−	−	−	+	−
Rectiflexibile	+	+	+	+	+	+	+	+	−	+	+	+	−	+
Spore surface	ND	ND	ND	Smooth[d]	Smooth[d]	Smooth[e]	Smooth	ND	ND	ND	Smooth[e]	Smooth[e]	Warty[e]	Hairy[e]
Color of aerial spore mass														
Flesh	+	−	−	−	−	−	−	−	−	−	−	−	−	−
Gray	−	−	−	−	−	−	−	+	−	−	−	−	−	−
Green	−	−	−	−	−	−	−	−	−	−	−	−	+	−
White	−	+	+	+	+	+	+	−	−	+	+	+	−	+
Growth on sole carbon sources														
Adonitol at 1% (wt/vol)	+	−	−	+	+	−	+	+	+	−	+	+	+	+
L-Arabinose at 1% (wt/vol)	+	+	−	+	+	+	−[f]	+	+	−	−	−	−	+
Dextrin at 1% (wt/vol)	−	−	−	−	+	+	+	+	+	−	+	−	+	+
D-Galactose at 1% (wt/vol)	−	−	−	−	+	−	−[f]	+	−	−	+	−	+	+
Glycogen at 1% (wt/vol)	−	−	−	+	+	+	−[f]	+	+	−	−	−	−	+
Lactose at 1% (wt/vol)	−	−	−	+	+	+	−[f]	+	+	−	+	+	−	+
Maltose at 1% (wt/vol)	+	−	+	+	+	+	−[f]	+	+	−	−	+	+	+
Mannitol at 1% (wt/vol)	+	−	−	+	+	+	+	+	+	−	+	+	+	+
L-Rhamnose at 1% (wt/vol)	−	−	−	+	+	−	+	+	+	−	−	+	−	+
Serine at 0.1% (wt/vol)	−	−	−	+	−	−	−[f]	+	−	−	−	−	−	+
Sodium propionate at 0.1% (wt/vol)	−	−	−	+	+	+	+	+	+	+	−	+	−	+
L-Tyrosine at 0.1% (wt/vol)	−	−	−	−	−	−	+	+	−	−	−	+	−	−
Growth at:														
pH 5	−	−	−	+	−	+	+	+	−	+	−	+	−	+
10°C	−	−	−	−	−	+	+	+	+	+	−	−	−	−

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TABLE 2—*Continued*

Test	*Amycolata autotrophica* K402	*Amycolata hydrocarbonoxydans* K428	*Amycolata saturnea* A195	*Amycolatopsis azurea* K114	*Amycolatopsis fastidiosa* K110[b]	*Amycolatopsis mediterranei* K98	*Amycolatopsis methanolica* 239	*Amycolatopsis orientalis* K99	*Amycolatopsis rugosa* K431	*Amycolatopsis sulphurea* K406	*Faenia rectivirgula* F1[b]	*Pseudonocardia thermophila* G37	*Saccharomonospora viridis* K73	*Saccharopolyspora hirsuta* K16
20°C	+	+	+	+	+	+	+	+	+	+	−	−	−	+
25°C	+	+	+	+	+	+	+	+	+	+	−	−	−	+
30°C	+	+	+	+	+	+	+	+	+	+	−	−	+	+
45°C	−	−	−	−	+	−	+	+	+	−	+	+	+	+
50°C	−	−	−	−	+	−	+	−	−	−	+	+	+	+
55°C	−	−	−	−	+	−	−	−	−	−	+	+	−	−
60°C	−	−	−	−	+	−	−	−	−	−	+	+	−	−
Growth in:														
Adenine at 0.4% (wt/vol)	−	−	−	+	−	−	+	+	+	−	−	−	−	+
Bismuth citrate at:														
1 μg ml^{-1}	+	−	+	+	+	+	+	+	+	+	−	−	−	−
10 μg ml^{-1}	+	−	+	+	−	+	+	+	+	+	−	−	−	+
100 μg ml^{-1}	−	−	−	+	−	−	+	+	+	+	+	+	−	+
Phenol at:														
100 μg ml^{-1}	−	−	−	+	−	+	+	+	+	+	+	−	−	+
1,000 μg ml^{-1}	−	−	−	−	−	−	−	−	−	−	−	−	−	+
Phenyl ethanol at:														
1,000 μg ml^{-1}	+	−	−	+	−	+	+	+	+	+	+	+	−	+
2,000 μg ml^{-1}	−	−	−	+	−	+	+	+	+	+	+	−	−	+
3,000 μg ml^{-1}	−	−	−	−	−	+	−	+	−	+	−	−	−	+
4,000 μg ml^{-1}	−	−	−	−	−	−	−	−	−	+	−	−	−	−
Potassium tellurite at:														
10 μg ml^{-1}	−	−	−	+	−	+	−	+	−	+	+	−	−	+
50 μg ml^{-1}	−	−	−	+	−	+	−	+	−	+	−	−	−	−
100 μg ml^{-1}	−	−	−	+	−	−	−	−	−	−	−	−	−	−
Sodium azide at 10 μg ml^{-1}	+	+	+	+	−	−	+	−	+	+	+	−	+	+
Sodium chloride at:														
3 μg ml^{-1}	−	−	−	+	+	+	+	+	+	+	+	+	+	+
5 μg ml^{-1}	−	−	−	−	−	+	+	+	+	+	+	+	+	+
7 μg ml^{-1}	−	−	−	−	−	−	−	+	+	+	+	+	+	−
10 μg ml^{-1}	−	−	−	−	−	−	−	−	+	−	+	+	−	−
13 μg ml^{-1}	−	−	−	−	−	−	−	−	+	−	−	−	−	−
Teepol at 100 μg ml^{-1}	−	+	+	+	−	+	+	+	+	+	−	−	−	+
Tetrazolium at:														
10 μg ml^{-1}	−	−	−	+	−	+	+	+	+	+	−	−	−	+
100 μg ml^{-1}	−	−	−	−	−	+	+	+	+	+	−	−	−	+
Crystal violet at 1 μg ml^{-1}	+	−	−	−	−	+	+	+	+	+	−	−	−	+
Thallous acetate at:														
10 μg ml^{-1}	+	−	+	−	−	+	−	+	+	−	−	+	−	+
100 μg ml^{-1}	+	−	+	−	−	+	−	+	+	−	−	−	−	+

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TABLE 2—*Continued*

Test	*Amycolata autotrophica* K402	*Amycolata hydrocarbonoxydans* K428	*Amycolata saturnea* A195	*Amycolatopsis azurea* K114	*Amycolatopsis fastidiosa* K110[b]	*Amycolatopsis mediterranei* K98	*Amycolatopsis methanolica* 239	*Amycolatopsis orientalis* K99	*Amycolatopsis rugosa* K431	*Amycolatopsis sulphurea* K406	*Faenia rectivirgula* F1[b]	*Pseudonocardia thermophila* G37	*Saccharomonospora viridis* K73	*Saccharopolyspora hirsuta* K16
Resistance to antibiotics														
Cephaloridine hydrochloride at:														
2 μg ml^{-1}	−	−	+	+	+	+	+	+	+	−	−	+	−	+
10 μg ml^{-1}	−	−	−	+	−	+	+	+	−	−	−	−	−	+
Demeclocycline hydrochloride at:														
2 μg ml^{-1}	−	−	+	+	+	+	−	−	−	−	−	−	−	+
8 μg ml^{-1}	−	−	+	+	−	+	−	−	−	−	−	−	−	+
Lincomycin hydrochloride at 10 μg ml^{-1}	+	−	−	+	+	+	+	+	+	+	+	+	+	+
Neomycin sulfate at:														
3 μg ml^{-1}	+	−	−	+	+	+	+	+	+	+	−	+	−	+
10 μg ml^{-1}	+	−	−	+	+	+	+	+	−	+	−	+	−	+
Oleandomycin phosphate at 2 μg ml^{-1}	+	−	−	+	+	+	+	+	+	+	−	+	−	+
Penicillin at:														
10 μg ml^{-1}	−	−	−	+	+	+	+	+	−	+	+	+	+	+
20 μg ml^{-1}	−	−	−	+	+	+	+	+	−	−	+	+	−	+
Rifampin at 10 μg ml^{-1}	−	−	−	+	+	+	−	+	−	−	−	−	−	+
Streptomycin sulfate at 16 μg ml^{-1}	−	−	−	+	+	+	−	+	−	−	−	+	−	+
Tobramycin sulfate at:														
1 μg ml^{-1}	+	−	+	+	+	+	+	+	−	+	−	+	−	+
8 μg ml^{-1}	−	−	−	+	+	+	−	+	−	+	−	+	−	+
Vancomycin hydrochloride at 0.025 μg ml^{-1}	−	−	−	+	+	+	+	+	+	+	−	+	−	+
Growth on sole carbon sources at 10 mM:														
Methanol	−	−	−	−	ND	ND	−	−	ND	ND	−	ND	ND	−
Ethanol	+	−	−	−	ND	ND	+	−	ND	ND	−	ND	ND	−
Propanol	+	−	−	−	ND	ND	+	−	ND	ND	−	ND	ND	−
Butanol	+	−	−	−	ND	ND	+	−	ND	ND	−	ND	ND	−
Methylamine	−	−	−	−	ND	ND	−	−	ND	ND	−	ND	ND	−
Ethylamine	+	−	+	−	ND	ND	+	−	ND	ND	−	ND	ND	−
Choline	−	−	−	−	ND	ND	+	−	ND	ND	−	ND	ND	+
Betaine	−	−	−	−	ND	ND	+	−	ND	ND	−	ND	ND	+
Sodium benzoate	−	−	−	−	ND	ND	+	−	ND	ND	+	ND	ND	+

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TABLE 2—*Continued*

Test	*Amycolata autotrophica* K402	*Amycolata hydrocarbonoxydans* K428	*Amycolata saturnea* A195	*Amycolatopsis azurea* K114	*Amycolatopsis fastidiosa* K110[b]	*Amycolatopsis mediterranei* K98	*Amycolatopsis methanolica* 239	*Amycolatopsis orientalis* K99	*Amycolatopsis rugosa* K431	*Amycolatopsis sulphurea* K406	*Faenia rectivirgula* F1[b]	*Pseudonocardia thermophila* G37	*Saccharomonospora viridis* K73	*Saccharopolyspora hirsuta* K16
L-Alanine	+	−	+	+	ND	ND	+	+	ND	ND	+	ND	ND	+
L-Arginine	+	+	+	+	ND	ND	−	+	ND	ND	+	ND	ND	+
L-Asparagine	+	−	+	+	ND	ND	+	+	ND	ND	+	ND	ND	+
L-Aspartate	+	−	+	+	ND	ND	+	+	ND	ND	+	ND	ND	+
L-Cysteine	+	+	+	−	ND	ND	−	−	ND	ND	−	ND	ND	−
L-Glutamate	+	+	+	+	ND	ND	+	+	ND	ND	+	ND	ND	+
L-Glutamine	+	+	+	+	ND	ND	+	+	ND	ND	+	ND	ND	+
L-Glycine	−	+	−	+	ND	ND	+	+	ND	ND	−	ND	ND	+
L-Histidine	+	−	−	+	ND	ND	−	+	ND	ND	+	ND	ND	+
L-Isoleucine	+	−	−	+	ND	ND	+	+	ND	ND	−	ND	ND	+
L-Leucine	+	+	+	+	ND	ND	−	+	ND	ND	−	ND	ND	+
L-Lysine	−	+	+	+	ND	ND	+	+	ND	ND	−	ND	ND	+
L-Methionine	−	−	−	−	ND	ND	−	−	ND	ND	−	ND	ND	−
D-Phenylalanine	−	−	−	+	ND	ND	+	+	ND	ND	+	ND	ND	−
L-Phenylalanine	+	+	−	+	ND	ND	+	+	ND	ND	+	ND	ND	+
L-Proline	+	+	+	+	ND	ND	+	+	ND	ND	+	ND	ND	+
L-Serine	+	−	−	+	ND	ND	+	+	ND	ND	−	ND	ND	+
L-Threonine	+	+	+	+	ND	ND	+	+	ND	ND	−	ND	ND	+
L-Tryptophan	−	−	+	+	ND	ND	−	+	ND	ND	−	ND	ND	−
L-Tyrosine	−	+	+	+	ND	ND	+	+	ND	ND	+	ND	ND	+
L-Valine	−	−	+	−	ND	ND	+	+	ND	ND	−	ND	ND	+

[a] All strains used glucose (1%, wt/vol), glycerol (1%, wt/vol), and sucrose (1%, wt/vol) as sole carbon sources and grew at pH 6 and 37°C, but none cleaved 4MU-β-L-fucopyranoside or CBZ-glycine-proline-7AMC nor did they grow in crystal violet at 10 μg ml^{-1}.

[b] Thermophilic strain.

[c] ND, Not determined.

[d] Data from Henssen et al. (25).

[e] Data from Williams et al. (52).

[f] Positive when tested at a carbon source concentration of 10 mM.

sensu Lechevalier et al. [32]); it lacks mycolic acids but has a fatty acid profile containing major proportions of 14-methylpentadecanoic acid (*iso*-C_{16}; 3.2% of total fatty acid composition), hexadecanoic acid ($C_{16:0}$; 24.3%), 14-methylhexadecanoic acid (*anteiso*-C_{17}; 21.8%), hexadecanoic acid ($C_{16:1}$; 11.6%), and an unidentified hydroxy fatty acid; and contains an isoprenoid quinone fraction composed of di- and tetrahydrogenated menaquinones with nine isoprene unites. These chemical markers serve to distinguish strain 239 from all other wall chemotype IV actinomycetes, including those belonging to the genus *Nocardia*, apart from those classified in the genus *Amycolatopsis* (9, 17, 25, 34).

Strain 239 has microbiological properties consistent with its assignment to the genus *Amycolatopsis* (34). It is an aerobic, mesophilic, gram-positive, non-acid-fast, nonmotile organism which forms an extremely branched substrate mycelium that bears aerial hyphae which show differentiation into squarish-to-oval spores on Czapeck Dox agar. Further, like the other *Amycolatopsis* sp. strains, it degraded casein, DNA, and tyrosine; cleaved 4MU-phosphate (acid), L-alanine-7AMC, isoleucine-7AMC, and tyrosine-7AMC; grew at pH 6.0, 20°C, and 37°C in lincomycin hydrochloride (10 μg ml^{-1}), neomycin sulfate (3 μg ml^{-1}), oleandomycin phosphate (2 μg ml^{-1}), and sodium chloride (3%, wt/vol); and used glucose (1%, wt/vol), glycerol (1%, wt/vol), sucrose (1%, wt/vol), and a range of amino acids (Table 2) as sole carbon and energy sources. In contrast, none of these strains degraded adenine or starch, grew in crystal violet (10 μg ml^{-1}), or cleaved 4MU-α-L-arabinopyranoside, 4MU-β-D-glucuronide, 4MU-β-L-fucopyranoside, or the carbobenzoyl N-protective group of amino acids (CBZ)-glycine-proline-7AMC. Further studies on additional representative strains are needed to realize the potential of rapid fluorogenic enzyme tests in the systematics of the family *Pseudonocardiaceae*.

The test strains can readily be separated from the type strains of established *Amycolatopsis* species by using a combination of biochemical, degradative, enzymatic, morphological, nutritional, and tolerance tests (Table 2). It is particularly interesting that *Amycolata autotrophica* K402 and strain 239 can be separated from the remaining organisms by virtue of the ability to grow on agarose mineral medium supplemented with ethanol, propanol, and butanol as sole carbon sources. Similarly, betaine and choline supported the growth of only *Saccharopolyspora hirsuta* K16 and strain 239. It should also be noted that strain 239 grows on methanol in mineral medium broth but failed to do so on the corresponding agarose medium.

It is evident from the chemical, molecular systematic, and microbiological data that *Nocardia* sp. strain 239 should be given species status in the genus *Amycolatopsis* Lechevalier et al. 1986. It is proposed that the organism previously known as *Streptomyces* sp. strain 239 (29–31) or as *Nocardia* sp. strain 239 (4, 5, 24) be classified in the genus *Amycolatopsis* as *Amycolatopsis methanolica* sp. nov.

Description of *Amycolatopsis methanolica* De Boer, Dijkhuizen, Grobben, Goodfellow, Stackebrandt, Parlett, Whitehead, and Witt sp. nov. (me.tha'noli.ca. M. L. n. *methanolicum*, methanol; M. L. fem. adj. *methanolica*, relating to methanol). Yellow vegetative growth carries abundant white aerial hyphae. Long chains of smooth, squarish spores (0.4 by 0.6 to 0.8 μm) in straight-to-flexuous chains formed on aerial hyphae when the organism is grown on Czapeck Dox agar. The spores are covered by a wall 0.04 to 0.06 μm thick. The chemical properties of the organism are described above, and many of the biochemical, degradative, enzymatic, nutritional, physiological, and tolerance features are given in Table 2.

Acid is formed from adonitol, L-arabinose, cellobiose, fructose, galactose, glucose, glycerol, mannose, rhamnose, ribose, salicin, sorbitol, trehalose, and xylose but not from dulcitol, erythritol, inositol, lactose, maltose, melibiose, raffinose, or sucrose. The organism, which is sensitive to lysozyme, decarboxylates acetic, benzoic, fumaric, α-ketoglutaric, lactic, malic, propionic, pyruvic, and succinic acids but not citric, formic, oxalic, or gluconic acid.

Growth occurs in mineral medium broth containing dimethylamine, trimethylamine, ethylamine, methanol, ethanol, 1-propanol, 1-butanol, 2,3-butanediol, acetone, betaine, choline, sarcosine, pectin, benzoic acid methylester, benzylamine, 3- and 4-hydroxybenzoates, 3,4-dihydroxybenzoate, phenylacetate, phenylacetaldehyde, phenyllactate, phenylpyruvate, 4-hydroxyphenylacetate, 4-hydroxyphenylpyruvate, D-phenylalanine, gentisate, and homogentisate as sole sources of carbon but not in methylamine, ethanolamine, propylamine, butylamine, methane, hexane, putrescine, benzene, toluene, or phenol; very sparse growth occurs in formaldehyde.

Isolated from a soil sample from New Guinea. Type strain: *Amycolatopsis methanolica* NCIB 11946.

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